Cultural characteristics.

Ordinary bouillon.—The colon bacillus renders the medium uniformly turbid, thickens it considerably, and gives it a gluish, gelatinous appearance. Sometimes, also, there is a superficial pellicle.

The typhoid bacillus produces a uniform turbidity. On shaking there is seen the play of colors characteristic of motile organisms. Upon the surface of the bouillon there is a pellicle, ordinarily thin, sometimes, however, quite thick.

Agar-agar slants.—The growth is rather sparse for both organisms; more abundant however for the typhoid than for the colon. It must be stated that the colon bacilli which give a pellicle in bouillon grow only upon the needle streak, while the bacilli which do not produce the pellicle in bouillon, give little or no growth along the streak of the needle. If we bear in mind that we almost always actively shake the flask before planting the plates, we arrive at a possible explanation of this singular peculiarity. The colon bacilli coming from the bottom of the flask render the bouillon turbid, but do not grow when planted along a streak on agar. The colon bacilli coming from the surface, give a pellicle in bouillon and grow well in streak on inclined agar-agar.

Gelatin.—Planted in tubes both organisms present a like appearance.

Characteristics of colonies on plates.

A. *Typhoid bacillus.*—Superficial colonies, as we have shown in the first part of our work, are excessively rare. We only, therefore, examine the deep colonies. These are small and bluish white.

B. *Colon bacillus.*—The colonies are either superficial or deep. The superficial are diffuse or globular; the deep are quite large, of a yellow brown color, or else are punctiform and bluish. Further details would be useless, as the most perfect description is not equal in value to the observation which anyone may make for himself.

Before leaving this subject of cultural characteristics we wish to draw particular attention to the two following points:

First. Colon and typhoid bacilli thus deprived of their characteristics by symbiosis at room temperature (from July 7 to October 29), no longer grow at a temperature of 37° C., and do not render bouillon turbid. On the contrary they grow energetically between 25° and 30° C.

Second. After a certain number of replantings on agar slants or in bouillon, even very much attenuated colon and typhoid bacilli regain their vitality, but never recover their distinctive properties (gas, indol, agglutination).

Bacteriologists are unanimous in agreeing that morphological and cultural characteristics are too indefinite to serve as a basis of differentiation between the typhoid and colon organism; is it therefore reasonable to ask it when it is a question of determining differences between organisms deprived of their specific characteristics?

If we judge, on the one hand, that Experiments VII and VIII have

given rise to serious doubts in favor of the typhoid origin of the bacilli tE1, tE2, tE3, tE4, and tE5, isolated from a mixture before the colon organisms had lost their properties, but that the organisms tE6, tE7, cE6, and cE7, isolated subsequently, have been classed by us as colon or typhoid based exclusively on cultural characteristics whose value we have just denied, we will have shown that it was indispensable to confirm our diagnosis, to see if there did not exist other means of permitting us to determine whether our organisms, motile and giving neither gas nor indol, and nonagglutinating, belonged to the typhoid group or the colon family.

The first proceeding consisted in endeavoring to restore to the organisms their colon and typhoid characteristics. Of very high scientific interest, this proceeding could hardly be employed in the current practice of bacteriological analysis of waters, which confronts us above all else in our present work; we have, therefore, reserved it for future investigation.

Another proof, of easier application, is given us by the property possessed by animals immunized by repeated injections of microbial cultures, of furnishing a serumagglutinative to the organism injected to the exclusion of all others. We therefore injected full grown guinea pigs with the bacilli tE1, tE2, tE3, tE4, tE5, tE6, tE7, cE6, and cE7, and we tried whether the serum agglutinated the colon bacillus or the typhoid bacillus isolated from stool No. 20, with which we had commenced work.

Operative procedure. We employed the subcutaneous method of injection, and introduced every two days, 2 c. c. of a two-day-old culture.

After the expiration of fifteen days an incision was made in the ear of the guinea pig, which enabled us to procure the serum whose agglutinating power we wished to determine. The agglutination experiments were made with young cultures (eighteen to twenty-two hours) in bouillon, as follows: With the aid of a Pasteur pipette, we took 9, 19, 29, etc., drops of the culture bouillon, and put them into watch glasses. With the same pipette, rinsed several times with distilled water, we added to the contents of the watch glasses a drop of the pure or diluted serum, the agglutinating power of which we desired to determine. After shaking, we left the watch glasses covered, at the temperature of the laboratory. After one and one-half hours of contact, the agglutination was examined by the naked eye and by the microscope. Every experiment was controlled by the preparation of a sample, under identical conditions, save the addition of the serum. This precaution was necessary, as we often observed that bouillon cultures, which formed no clumps in tubes, often coagulated spontaneously in the control preparations. Another source of error against which one must be on his guard is that the normal serum of the guinea pig almost always agglutinates the typhoid bacillus in a dilution of 1–5

to 1–10, rarely 1–20. It only exceptionally agglutinates the colon bacillus in 1–10 to 1–20.

Experiment I.—Injection of tE_1—that is to say, of the typhoid bacillus of stool No. 20, isolated from the mixture of colon and typhoid bacilli after five days of symbiosis.

For this experiment we continued the injection every two days for nine weeks, in order to learn the number of injections necessary to bring the serum to its maximum of agglutinating power. The results were as follows:

Bacilli experimented with.	Agglutinating power, after—				
	Fifteen days.	Three weeks.	Five weeks.	Seven weeks.	Nine weeks.
t. stool 20	1/50	1/4000	1/8000	1/8000	1/8000
tE_1 (injected)	1/50	1/800	1/800	1/1000	1/2000
tH_1 (fromacid mixture)	1/50	1/800	1/800	1/800	1/1000
c. stool 20	1/10	1/10	1/10	1/10	1/10
	None.	None.	None.	None.	None.

Conclusions.—1. The guinea pig injected with tE1 furnished a serum which agglutinated the typhoid bacillus from stool No. 20 to the exclusion of the colon bacillus from the same stool; consequently the bacillus tE1 is, therefore, a typhoid bacillus from stool No. 20, which symbiosis with the colon bacillus had deprived of its power of reaction to the agglutinins in the experimental antityphoid serum.

2. The serum agglutinated the typhoid bacillus from stool No. 20 in a much higher dilution than the bacillus tE1, from the injection of which it was prepared.

3. The agglutinating power reached its maximum five weeks after the first injection of the organism from stool No. 20. From this time on further injections had no influence on the agglutinating power. Toward the bacillus itself injected on the contrary, the maximum power was obtained only after the expiration of nine weeks from the first injection.

NOTE.—An experiment with the bacillus tH1 gave identical results.

NOTE.—In this first experiment we have given the agglutinating results for various specimens of typhoid bacilli. We believe it would be a departure from the present subject if in the experiments which follow we should deal with the agglutination of bacilli other than t. s. 20, and c. s. 20. These are the only ones in point of fact which present any real importance from the point of view "of the antagonism between the bacillus typhoid and the bacillus coli," which forms the subject of this second part of our work. These are the only ones the agglutination of which could permit us to solve the question which we have proposed at the beginning of this chapter, "to determine whether the motile organisms, giving neither gas nor indol, and not aggutinated by serum, belong to the colon or the typhoid group of organisms isolated from stool No. 20."

Experiment II.—In this we have employed bacillus tE5, isolated from the mixture on the eighty-second day of symbiosis. The colon bacillus

s. 20 no longer gave indol, but fermented lactose. We proceeded as in Experiment I.

Bacilli injected.	Agglutinating power after—				
	Fifteen days.	Three weeks.	Five weeks.	Seven weeks.	Nine weeks.
t. s. 20	$\frac{1}{50}$	$\frac{1}{500}$	$\frac{1}{500}$	$\frac{1}{500}$	$\frac{1}{500}$
c. s. 20	None.	None.	None.	None.	None.

Conclusions.—1. The guinea pig injected with tE5 furnished a serum which agglutinated the typhoid bacillus of stool No. 20 to the exclusion of the colon bacillus from the same stool. Consequently the bacillus tE5, isolated from the mixture on the eighty second day of symbiosis, is a typhoid bacillus from stool No. 20, deprived of its sensibility to agglutinins by said symbiosis.

2. Since the bacilli tE1 and tE5, isolated from the mixture on the fifth and eighty-second days, respectively, of symbiosis, are both typhoid bacilli, deprived of their sensibility to agglutinins, the bacilli tE2, tE3, and tE4, found in the same mixture between the fifth and the eighty-second days, are also samples of the same bacillus.

We have thus shown in an indubitable way that the bacilli tE1, tE2, tE3, tE4, and tE5, isolated from the mixture between the third and eighty-second day of symbiosis, *when the bacillus coli was not deprived of its distinctive powers, are representative of the bacillus t. s. 20.*

Is it the same with the bacilli tE6 and tE7 found in the mixture on the one hundred and second and one hundred and twenty-first days of the symbiosis, when the bacillus coli *was* deprived of its distinctive characteristics? The following experiments will permit us to answer the question:

Experiment III.—We employed the bacillus tE6, isolated from the mixture on the one hundred and second day of symbiosis. At this time the bacillus coli of stool No. 20 was lacking in its characteristic properties (gas, indol, etc.).

The experiment shows that the two bacilli submitted to test show no reaction and the agglutinating power of the serum is "nil" after fifteen days, and three, five, seven, and nine weeks.

Conclusion.—The serum of the guinea pig injected with bacillus tE6. does not agglutinate the bacillus t. s. 20, and is equally without effect on the bacillus c. s. 20. We can, therefore, feel no certainty as to the colon or typhoid origin of bacillus tE6.

Experiment IV.—We injected the bacillus cE6 in order to try to determine its nature.

Bacilli injected.	Agglutinating power, after—				
	Fifteen days.	Three weeks.	Five weeks.	Seven weeks.	Nine weeks.
t. s. 20	nil	nil	nil	nil	nil
c. s. 20	$\frac{1}{50}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$

Conclusions.—1. The serum of the guinea pig injected with cE6, agglutinating the bacillus coli from stool No. 20 to the exclusion of the typhoid bacillus from the same stool, we are justified in considering cE6 as being the colon bacillus from stool No. 20, deprived of its properties (gas, indol, etc.) by the symbiosis with the typhoid bacillus from the same stool.

2. Since cE6 is the colon bacillus from stool No. 20, deprived of its characteristics, we believe it must be admitted that the bacillus tE6, the origin of which could not be determined by Experiment III, is really the typhoid bacillus from stool No. 20. In fact the colonies of bacilli cE6 and tE6 were found on the same gelatin plate, and were only labeled thus because they presented different appearances.

Experiments V and VI.—With the bacilli cE7 and tE7 we injected 2 guinea pigs. These organisms were isolated after one hundred and twenty-one days of symbiosis.

These pigs yielded a serum (even after seven and nine weeks) devoid of any agglutinating power upon the typhoid or colon bacilli of stool No. 20 after one hundred and twenty-one days of symbiosis.

Conclusion.—It was, therefore, impossible to determine if the organisms isolated from a mixture of typhoid and colon bacilli, from stool No. 20, belonged to the typhoid or colon group after this period of symbiosis.

The study of the agglutinating properties of the serum of guina pigs which were injected with bacilli tE1, tE2, tE3, tE4, and tE5 permitted us to demonstrate that these organisms were the descendants of the typhoid bacilli of stool 20, because we had preserved in our collection the Eberth bacillus isolated from stool 20, which we had used as a standard. If, however, we were to find ourselves confronted by organisms, of which we did not know the genesis, as might well happen in the bacterial analyses of water, where stock organisms are wanting, how would we be able to determine whether organisms similar to tE1, tE2, etc., which are motile, which do not give gas or indol and which are not agglutinated by antityphoid serum, are typhoid or colon?

It has been known for a long time, and we have just demonstrated anew, that the repeated injection of a guinea pig with a typhoid bacillus gives a serum which agglutinates an authentic typhoid bacillus. Is the converse of this proposition true? Does a serum agglutinative to authentic typhoid organisms, always follow the injection of a typhoid bacillus? This would seem evident, *a priori*; it is in fact the attitude of the clinician when he makes the sero-diagnosis of typhoid fever. He admits that there is a tox-infection by the Eberth bacillus when the serum of the patient agglutinates a true typhoid culture under the required conditions. Consequently, an unknown bacillus, presenting the cultural characteristics of the Eberth bacillus, should be considered as the typhoid bacillus if the serum of a guinea pig injected with it agglutinates authentic typhoid organisms in a sufficient degree of dilution.

We therefore injected bacilli tE1, tE2, tE3, and tE4, into guinea pigs

and tried after fifteen days the agglutinating power of the serum, using the bacillus typhoid of Liége as a standard.

Bacilli injected.	Agglutinating power after fifteen days.	Observations.
tE1	$\frac{1}{40}$	Isolated from neutral mixture after five days.
tH1	$\frac{1}{40}$	Isolated from acid mixture after five days.
tE3	$\frac{1}{40}$	Isolated from neutral mixture after forty-six days.
tH3	$\frac{1}{40}$	Isolated from acid mixture after forty-six days.
tE4	$\frac{1}{30}$	Isolated from neutral mixture after fifty-eight days.
tH4	$\frac{1}{30}$	Isolated from acid mixture after fifty-eight days.
tE5	nil	Isolated from neutral mixture after eighty-two days
tH5	nil	Isolated from acid mixture after eighty-two days.

Conclusions.—The agglutination by the serum of the guinea pig injected with the bacilli tE1, tE2, tE3, tE, and tE4, is a process which can teach us nothing as to the nature of bacillus tE5; it is uncertain for tE4; it is a rapid and sure method for bacilli tE3. tE2, and tE1.

In fact, the guinea pigs injected with these give a serum which in $\frac{1}{40}$ agglutinates the typhoid bacillus of Liége. This dilution is sufficient, for it is fully equal to the dilution required by any clinician to settle the diagnosis of typhoid fever by the serum reaction.

To confirm this opinion we injected into the subcutaneous tissue of guinea pigs bouillon cultures of typhoid bacilli, other than that of Liége, and after fifteen days we determined the agglutinating power of the serum against the bacillus of Liége:

Bacilli injected.	Agglutinating power after fifteen days.	Observations.
t. s. 20	$\frac{1}{50}$	
t Ghent	$\frac{1}{30}$	Died on eighth day of experiment.
t. s. 22		Died day after injection.
t. s. 15		Do.

Conclusions.—The greater part of the guinea pigs injected died before the end of the experiment, with a general dissemination of the typhoid organism in all the organs. However, the pig which received bacillus t. s. 20 gave, after fifteen days, a serum agglutinating in dilution of $\frac{1}{50}$, which is a strength closely approaching that obtained by the inoculation of bacilli tE1, tE2, and tE3.

Before drawing these experiments to a close it was indispensable to try whether the serum of guinea pigs injected with colon bacilli of various origins could agglutinate the typhoid bacillus of Liége. The results follow in the subjoined table:

Bacilli injected.	Agglutinating power after fifteen days.	Observations.
Colon, Ghent	nil	Pig died on twelfth day.
s20	nil	Pig died on third day.
s205	nil	
C1	nil	
C2	nil	
C3	nil	
C4	nil	
C5	nil	

Conclusions.—The serum of a guinea pig injected with the colon bacillus does not agglutinate the typhoid bacillus of Liége. Consequently, the study of the agglutinative power of the serum of a guinea pig in which is injected for fifteen days (every two days) 2 c. c. of a forty-eight-hour-old culture of typhoid constitutes a practical and sure method for determining the typhoid nature of certain Eberth bacilli which have lost their sensibility to the agglutinins of an experimental antityphoid serum. Other bacilli which are not agglutinated, and which are, however, the bacilli of Eberth, escape our observation by the methods of which we are in possession at the present time.

General conclusions.—Having thus finished the second part of our work, we believe ourselves justified in formulating the following conclusions:

1. The conception of the destruction of the typhoid bacillus by the colon bacillus, sustained by Wathelet in the laboratory of Malvoz, is weakened by our researches upon the antagonism of these two organisms. This author did not find the typhoid bacillus in his mixtures, not because the typhoid bacillus no longer existed there, but because it was placed under conditions unfavorable for its discovery.

2. Symbiosis may profoundly modify the properties of the two organisms; the typhoid bacillus losing its sensibility to agglutinins, and the colon bacillus being deprived of its properties of gas and indol production, etc.

3. Very different in the beginning from the typhoid colonies certain deep-seated colon colonies approach them insensibly in size and appearance from the third to the fourth week. They are then truly blue in color, while the typhoid colonies are bluish white.

4. The attenuation of the vital energy of the organisms is manifested not only by a diminution of growth of colonies, but also by a lateness of their appearance. This is especially true for the typhoid colonies, which from the third to the fourth week only become visible about the

fifth day, while in the beginning they appear on the second day after planting.

5. If the agglutination of bacilli, presenting the typhoid characteristics, by an experimental typhoid serum in high dilution, is a means sufficient to authorize us to consider it a typhoid, the absence of this sensibility does not permit us to reject it as not belonging to the typhoid group.

6. Bacilli possessing the attributes of the typhoid bacillus, which are not agglutinated by the experimental antityphoid serum, ought to be considered as typhoid if a guinea pig into which it is injected in doses of 2 c. c. every two days for fifteen days furnishes a serum at the end of that period, which agglutinates true typhoid bacilli in a dilution of $\frac{1}{40}$.

7. There may exist true typhoid bacilli, nonagglutinable by antityphoid serum, whose typhoid nature it is impossible to determine either by the process given above or by any other diognostic means of which we are now in possession.

[Reports to the Surgeon-General United States Marine-Hospital Service.]

Smallpox in Girard and Phœnix, Ala., and Columbus, Ga.

MOBILE, ALA., *December 31, 1900.*

SIR: I have the honor to state that in conformity to Bureau orders of December 23 and December 24, directing me to proceed to Columbus, Ga., and confer with health authorities there as to best means of protection against smallpox in Girard and Phœnix, Ala., I left Mobile on the night of the 25th, arriving in Columbus on the morning of the 26th. I called on the city physician, who accompanied me to the mayor's office.

I was informed that there was no smallpox known to exist in Columbus outside of the smallpox hospital, some 10 or 12 cases being confined there, but the situation was thought, and afterwards found to be, much more serious in both Girard and Phœnix. The mayor of Columbus stated that he would be very glad to have the Service advise the local authorities as to the best methods of keeping the disease from reaching Columbus from the 2 cities named. I deemed it best to wait until I knew something about the conditions in Girard and Phœnix before giving advice; I therefore wired Dr. W. H. Sanders, State health officer of Alabama, asking when he would be in Girard. He replied that he would be there the next day (Thursday). On Thursday I met Dr. Sanders in Girard. He accompanied me to Columbus where we had a talk with the mayor; we then visited Phœnix, Lee County, Ala., where we saw the mayor and some of the local physicians. They reported that there were some 12 or 15 cases of smallpox in the city. One of the physicians reported having seen 5 new cases that day. Very little was being done in the way of vaccination or isolation to control the disease. A lack of funds was given as a reason for this. Dr. Sanders gave them some wholesome advice and positive instructions, which if properly carried out, will no doubt lead to great improvement. The disease is confined, I was told, almost entirely to the white people.

From Phœnix we again visited Girard. The conditions there are similar to those in Phœnix, with the exception that there are many more cases in Girard; there are also 15 cases among the negroes in Sugartown, a suburb. Girard is in Russell County, and we were informed